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ANNEX

CLAIMS

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1. Polypeptides having antifreeze activity which can be obtained from carrots and which have an apparent molecular weight on SDS-PAGE of 36 kDa and isoforms or derivatives thereof which still possess antifreeze activity.

Sub C

2. Polypeptides having antifreeze activity comprising one or more fragments (A-E) of the amino acid sequence as follows:

(A) LEU-PRO-ASN-LEU-PHE-GLY-LYS

(B) ILE-PRO-GLU-GLU-ILE-SER-ALA-LEU-LYS

(C) LEU-THR-X-LEU-ASP-LEU-SER-PHE-ASN-LYS

(D) SER-LEU-ARG-LEU-SER-SER-THR-SER-LEU-SER-GLY-PRO-
VAL-PRO-LEU-PHE-PHE-PRO-GLN-LEU-X-LYS

(E) X-X-GLY-VAL-ILE-PRO-X-GLN-LEU-SER-THR-LEU-PRO-
ASN-LEU-LYS

and isoforms or derivatives thereof which still possess antifreeze activity.

AMENDED SHEET

3. Polypeptides having antifreeze activity comprising the fragments (A-E) of claim 2.
4. ~~Polypeptides having antifreeze activity having an amino acid sequence as represented in Listing 1 and isoforms and derivatives thereof which still possess antifreeze activity.~~
5. An isolated nucleic acid sequence encoding the ~~antifreeze polypeptide of one or more of claims 1-4~~ ^{Claim J} and alleles thereof encoding polypeptides which still possess antifreeze activity.
6. An isolated nucleic acid sequence corresponding to the gene sequence of Listing I and alleles thereof encoding polypeptides which still possess antifreeze activity .
7. Method of obtaining polypeptides according to one or more of ~~claims 1-4~~ ^{Claim I} whereby the polypeptide is isolated from cold-acclimatized carrots.
8. Method of obtaining polypeptides according to one or more of ~~claims 1-4~~ ^{Claim J}, whereby the polypeptide is expressed by a genetically modified organism.
9. Method according to claim 8, whereby the organism is a micro-organism, a plant or a cell culture.

- a*
10. An antibody capable of specifically binding the polypeptide of *Claim* 1, 2, 3 or 4.
- Sub C3* *>*
11. A polypeptide which has antifreeze activity that is immunologically related to the polypeptide of claim 1, 2, 3 or 4 as determined by its cross reactivity with an antibody of claim 10.
- a*
12. Food product comprising a polypeptide of *Claim* 1, 2, 3, 4 or 11 with the proviso that the food product is not a carrot.
- a*
13. Food product of claim 12 being a frozen confectionery product or a frozen vegetable.
- a*
14. Method of producing a food product comprising an antifreeze polypeptide according to *one or more claims* 1, 2, 3 or 4, comprising the steps of
- (a) adding to the food product a composition comprising said antifreeze polypeptide; or
- (b) in situ production of said antifreeze polypeptide.
- Sub C4* *>*
- a*
15. Use of the polypeptide of *Claim* 1, 2, 3 or 4 for increasing the frost tolerance of plants.

16. Micro-organisms, cell line or plant capable of expressing the polypeptide of claims 1, 2, *3 or 4, with the proviso that the plant is not an unmodified carrot plant.

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The above data provides the proof that the protein purified from carrot and the corresponding cDNA represent an active AFP.